POC

Made with Benchling

Project: Undergrad

Authors: Azzurra Laura De Pace

Dates: 2016-06-27 to 2016-09-01

MONDAY, 27/6

Team members: Pepy, Brendan

Annealing of word primers

1. Prepared Annealing buffer (10 mM Tris-Hcl, 50mM NaCl, 1mM EDTA) 100mL with pH = 7.44
2. Resuspended each primer with set buffer volume, using O.D. values from IDT to produce 100µM solutions for each primer.
3. Mixed 1µg of each primer pairs of ‘good’ and ‘day’ in 2 separate Eppendorf tubes (0.704 µL of each primer solution) and filled up each tube to 50 µL with the prepared buffer.
4. Both word tubes were heated for 2 min at 92 C and left to cool at room temperature.
5. The prepared words solutions were stored in freezer

TUESDAY, 28/6

Team members: Pepy, Brendan

Inserting words into vectors

Prepared stock solutions of **BBa_P10500** plasmid and 'good' 'day' words at **5fmol/µL.**

1. Prepared stock solutions of T4 ligase at 20U/µL
2. Prepared word-vector-insertion mixtures in PCR tubes for each word prepared on 27/06.
   Mixtures were prepared using CIDAR MoClo Protocols for creating basic parts:

- 2µL of BBa_P10500 plasmid stock solution (10fmol)
- 2µL of 10x Promega Ligase buffer
- 1µL of BsmBI (10U/µL)
- 1µL of T4 Ligase (20U/µL)
- 12µL of d.H_2O added

'Good' and 'Day' mixtures were prepared
where total volume of each mixture - 20 µL

4. Used PCR cycle protocol from Jon's lab to incubate a one pot reaction of cleavage/ligation in BBa_P10500 plasmid:

clipboard_2016-06-28_17:51:54.png

Rapid protocol	Step 1	37°C	20
Ideal for new basic parts	Step 2	37°C	1.5
	Step 3	16°C	3
	Cycle 2-3	x5-10	
	Step 4	50°C	5
	Step 5	80°C	10
	Total time		37.5-60

At the end of the programme DNA is kept at $4^{o}C$
Programme was named PEPY at the middle PCR Thermocycler

Team members: Rosie, Alex and Azzurra

Transforming TOP10 cells with BBa_P10500

1. a transformation was performed using the standard protocol and 0.5 µL of the OG 162.5 nmol/mL solution

WEDNESDAY, 29/6

Team members: Pepy

Transforming TOP10 cells with BabbleBricks

1. A transformation was performed using the standart protocol and 20µL of each BabbleBrick prepared on 28/06
   NOTE: contaminated LB was used instead of SOC medium

Inoculation of cells from yesterday

1. An inoculation of 1 colony in LB and chloramphenicol solution of the transformed cells was performed

Inserting words into vectors

1. 'Good' and 'Day' BabbleBrick assembly protocol from 28/06 was repeated with both words solutions and PCR cycle was left overnight

THURSDAY, 30/6

Transformation of competent cells with 'good' Babblebrick

1. Followed transformation protocol using 20µL of 'good' Babblebrick prepared from yesterday.

Miniprep of pUdp2/P10500 plasmid backbone

1. Miniprep protocol was followed and a stock solution was prepared.

Prepared master mix for future Babblebrick assembly reactions:

- 12 µL H_2O
- 2 µL word
- 2 µL plasmid x50
- 2 µL ligase buffer
- 1 µL BsmBI (10 000U/mL)
- 1 µL T4 ligase stoch (20 000U/mL)

So the master mix doesn't have DNA and contains:

- 600 µL H_2O
- 100 µL ligase buffer
- 50 µL BsmBI (10 000U/mL)
- 50 µL T4 ligase (20 000U/mL)

Inoculation of pUdp2 plasmid culture and 'good' and 'day' Babblebricks screened on 29/06
White colonies were observed on both screening plates.

1. 13 inoculation mixtures were prepared (5 µL of 34mg/mL Chloramphenicol and filled up to 5mL with LB)

2. 'good' 100 µL Colonies 1,2,3 = 3 tubes
3. 'good' rest Colonies 1,2,3 = 3 tubes
4. 'day' 100 µL Colonies 1,2,3 = 3 tubes
5. 'day' rest Colonies 1,2,3 = 3 tubes
6. pUdp2 culture = 1 tube

7. Colonies taken from agar plates have been labelled
8. Incubated at 37 C overnight

Annealing of Anchor oligo

- 100 µL of each primer stock solution (100µM)
- 800 µL of annealing buffer
- heated for 2 min at 92 C and left to cool at room temperature.

Producing a 10 µM stock solution of anchor

FRIDAY, 1/7

Miniprep of 'good' and 'day'

- Followed protocol only for 'good' and 'day' 100 µL colonies
- Rest of the colonies innoculated were frozen

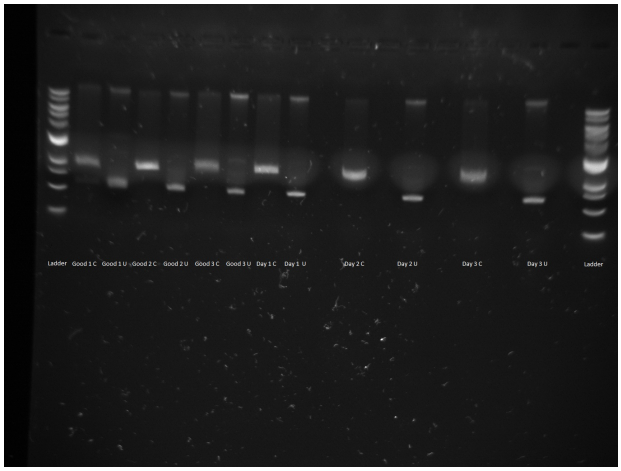
EcoRI digestion of 'good' 'day' plasmids

- 2 µL of DNA miniprep gives you 100-200 ng of DNA
- Colonies 1,2,3 from 100µL cultures for both words were used for cutting
- The protocol for digestion was followed, where EcoRI and CutSmart Buffer were used
- each Babblebrick was incubated for digestion for 2 h at 37C

Gel electrophoresis of 'good' 'day'

- Followed gel preparation protocol for 0.8% agarose solution
- Colonies 1,2,3 from 100µL cultures were prepared uncut
- 20µL of each solution was run on the gel:

01_07 digestion of babblebricks.tif



Beads testing

1. 25 µL of beads put in 7 eppendorf tubes
2. 100µL of anchor solution diluted 1:2 5 times using control buffer (TE + NaCl = 10mM Tris 50 mM NaCl)

100 µL used than 50 µL (filled to 100µL with buffer), 25 µL, 12.5 µL and so on

1. Liquid was removed while holding the beads on the tube with a small magnet
2. Bead solutions were washed three times (2 with control buffer and 1 with water) - this step removes the beads buffer.
3. 12.5 µL of anchor solution was added to each bead solution
4. End solutions were shook for 10 min
5. Tubes were spun
6. The supernatant was removed and collected for measurement
7. Then the cycle was repeated by washing the beads 3 times (step 2)
8. 100 µL of buffer was added, solutions were "melted" at 50 C for 2 min.
9. the supernatant was quickly removed and collected for measurement

Problems observed:

- During the first washing, removing the beads from the supernatant and discarding them was hard, inneficient and time consuming. This causes some beads to be discarded together with the supernatant. For some tubes a small quantity of supernatat was left causing volume problems in the nex step. Therefore, we couldn't collect the first supernatat. This procedure might work well using a stronger magnet and always spinning the tubes

- When melting the tubes, separating the supernatant was difficult. Having 7 tubes they were left on the bench for longer and might have re-annealed. Therefore, we repeated the melting step in pair of two tubes per time to prevent them from cooling down. Heather suggested using the magnet directly, instead of spinning the tubes. Unfortunately, in some tubes there were beads left (4 & 6)
- Procedure must be repeated using stronger magnet, spinning, working fast (removing the supernatant as fast as possible)

MONDAY, 4/7

Measurements of the anchor and the beads with a spectrophotometer

A=ε c L ε (ds DNA) = 0.020 µg/mL ε(ss DNA) = 0.027 µg/mL

Samples were diluted (100 µL sample + 900 µL buffer) to reach a volume of 1 mL for spectrophotometer readings
Plain annealing buffer was used as a blank reference of the readings.
The following three tables group together the experimental data for the anchor beads-binding assay:

Table1

	A	B	C
1	Tube	1s'	2s'
2	260 nm (DNA)	0.058	0.003
3	280 nm (protein)	0.015	0.008

Table1.1

	A	B	C	D
1	Tube	3s'	4s'	5s'
2	260 nm (DNA)	0.016	0.009	0.005
3	280 nm (protein)	0.014	0.007	0.006

Table1.2

	A	B	C
1	Tube	6s'	7s'
2	260 nm (DNA)	0.005	0.001
3	280 nm (protein)	0.006	0.006

TUESDAY, 5/7

PCR of 'good' 'day' plasmids

1)Make 10mM dNTP stock:

- 10 µL dATP
- 10 µL dTTP
- 10 µL dCTP
- 10 µL dGTP
- 60 µL dH_2O

100 µL in 0.5 eppendorf

2)Make PCR mix in 0.5 eppendorf

- 97.5 µL Nuclease free water
- 30 µL 5x Q5 reaction buffer
- 7.5 µL 10 µM forward primer (UF2)
- 7.5 µL 10 µM reverse primer (UR)
- 3 µL 10 mM dNTP

145.5 µL mix

3) Add to each PCR tube 'good' and 'day'

- 48.5 µL PCR mix
- 1.0 µL of each DNA template (used from 01/07 miniprep) 1.0 µL of 'day' miniprep and 1 µL of 'good'miniprep separately
- 0.5 µL DNA pol

4) Cylcle for 500 kb PCR fragment (Saved on JCMBI --> iGEM as PCR UG):

Table2

	A	B	C	D
1	Stage	Cycles	Temp (C)	Time(s)
2	1	1	98	30
3	2	30	98	10
4			66	30
5			72	15
6	3	1	72	120
7	Hold	at	4 C	

Before placing the tubes in the PCR machine, they were centrifuged for 30s at 13000 rpm for mixing

Repeating bead-anchor binding efficiency assay

- Followed the protocol from 01/07,(binding assay 1) where an extra dilution at the end was performed
- Used a magnetic rack instead of a magnet
- Spectrophotometer readings of 1/10 dilutions (Buffer - tube #9 was used as blank):

Table3

	A	B	C	D	E	F	G	H
1	Tube	1	2	3	4	5	6	7
2	260nm (DNA)	0.14	0.075	0.108	0.069	0.032	0.056	0.139
3	280nm (protein)	0.2	0.091	0.062	0.096	0.034	0.068	0.168

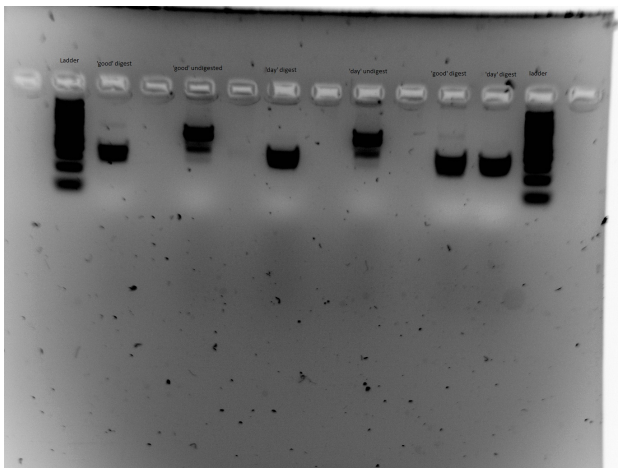
Measurements attempted to be repeated with a new spectrophotometer but since values didn't make sense, machine was concluded to be broken.

WEDNESDAY, 6/7

Gel electrophoresis of 'good' and 'day' digests with a low MW ladder

- ladder taken from Chris French
- Followed protocol to prepare a 0.5 L 0.8% (weight/volume) agarose gel
- Followed protocol to prepare a 0.1 L 4 % from Interlab page 1
- Gel set up with low MW ladder and run at a low voltage (60-70mV) for approx 1 hour
- gel was run backwards for some time, then was corrected
- eletrophoresis machine was dripping

06_07 digestion of babblebricks small ladder.tif



Word fragments were not observed.

Repeated anchor-bead binding efficiency assay

- Starting from the 10μM stock solution of anchor, eight 100μL anchor solutions were prepared where 2/3 dilutions were performed.

The following two tables group together the experimental data:

Table4

	A	B	C	D	E
1	Tube (100μL)	1	2	3	4
2	Concentration	8.1μM	5.4μM	3.6μM	2.4μM

Table4.1

	A	B	C	D	E
1	Tube (100μL)	5	6	7	8
2	Concentration	1.6 μM	1.06 μM	0.71 μM	0.474 μM

- Beads binding efficiency protocol 1 was followed where 12.5 μL of each anchor solution was used.

FRIDAY, 8/7

BsaI digestion of 'Good' and 'Day'

Digested PCR products from 6/7/16 'good' and 'day'. The protocol used was taken from the BsaI-HF card.

Gel preparation

100 ml of 40% agarose gel were prepared following the same protocol as on 6/7/16. Run at 90 mv for 45 min.

Beads binding assay using different melting temperatures and the original stock concentration of anchor (10 μl)

The protocol was the same as the one used on the 1/7/16.

Table5

	A	B	C	D	E	F	G	H	I	J
1	Melting temperature (C)	room temperature (around 25)	45	50	55	60	60	65	70	75
2	Concentration (ng/μl)	2.5	1.5	2.3	2	2.3	2.3	2.7	2.7	3.9

MONDAY, 11/7

Assambly Assay

1. add 25 μl of beads
2. wash the beads three times like in the beads binding assay
3. incubate while shaking for 30 min

4. for test 1 and 2 the supernatant was not collected before starting the second washing cycle. Therefore, one extra wash with annealing buffer was performed.
5. 20 µl of 10 uM 'good AB hang' was added together with 1 µl of Ligase and the mixture incubated for 30 min
6. Repeat step 2
7. 20 µl of 10 uM 'day BA hang' was added together with 1 µl of Ligase and the mixture was incubated for 30 min while shaking
8. repeat step 2
9. 12.5 µl of 100 uM terminator was added with 1 µl of T4 ligase stock and shaked for 30 min.

TUESDAY, 12/7

Ladder Stock preparation

Intial stock solution = 500 µg/ml
Recipe for 20 µg/ml
-20 µl ladder
-80 µl dye
-300 µl H2O

Running of the assembly made on 11/7

Gel picture in the book.

PCR preparation of 'Good' and 'Day'

Mastermix recipe enough for 3 reactions:
- 30 µl Q5 polymerase buffer
- 3 µl dNTPs
- 1.5 µl polymerase
- 97.5 µl water
In each tube:
-2.5 µl of each primer
-1 µl of either 'good' and 'day' miniprep

WEDNESDAY, 13/7

Gel of PCR product from 12/7

Gel picture printed and in the book (not saved on the usb)

MONDAY, 18/7

Annealing of primers words

Annealing of primers Protocol (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/etr-pMAZ6X2t-annealing-of-primers/edit)

TUESDAY, 19/7

Insertion of words into P10500

- 5 fmol/µl stock solutions of 'there AB', 'to BA', 'remain AB', 'Address00 BA', '0 AB', '1 AB' and '2 BA' were prepared from stock with concentration of 1.44 µM
- Using 5 fmol/µl of P10500 stock 'insertion PCR mixtures' were prepared using the protocol from 28/06.
- Heat block and water bath were used instead of PCR machine cycle for the incubation cycle
- Tubes were frozen and labelled as 'inserts'

Protocol: Word insertion protocol (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/etr-6jPrhEXv-word-insertion-protocol-from-jons-lab/edit)

WEDNESDAY, 20/7

PCR of 'Good' and 'Day'

Transformation of word inserts

Transformation of competent cells (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/prt-VAC2Frf2-transformation-of-competent-cells-/edit)

Clean up of 'Good' and 'Day' PCR from 19/7 PCR with the new Nucleotidal Removal Kit

Digetsion of cleaned PCR products

Digestion of plasmid for cloning (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/prt-YaxGjRxZ-digestion-for-cloning/edit)

2% agarose gel of G and D PCR, G and D cleaned PCR, G and D digested

gel (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/prt-nWZtMlPg-gel-electrophoresis/edit)

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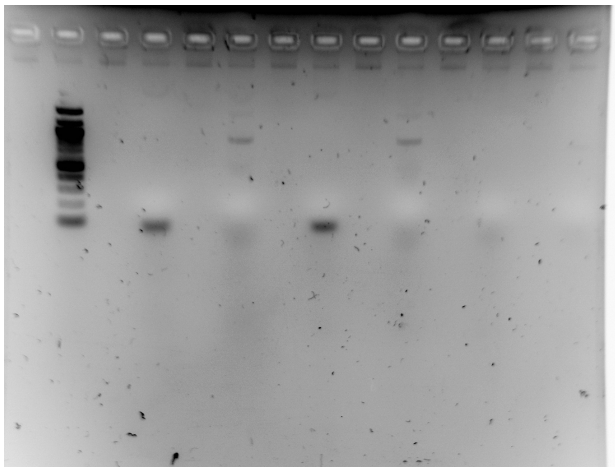

Set up of a gradient PCR for 'Good' and 'Day'

THURSDAY, 21/7

Inoculation of BabbleBricks: 'to BA', 'remain AB', '1 AB' and '2 BA'

Insertion and transformation of BabbleBricks: 'there AB', '0 AB' and 'address00 BA'

Word insertion protocol (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/etr-6jPrhEXv-word-insertion-protocol-from-jons-lab/edit)

Transformation of competent cells (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/prt-VAC2Frf2-transformation-of-competent-cells-/edit)

Gel of gradient PCR done on 20/7

Picture in the labbook

FRIDAY, 22/7

PCR of 'Good' and 'Day' with DMSO

it was only succesful for Good, no picture exists

PCR of 'Good' and 'Day' using Fusion Polymerase

MONDAY, 25/7

Gel of PCR with Fusion Polymerase

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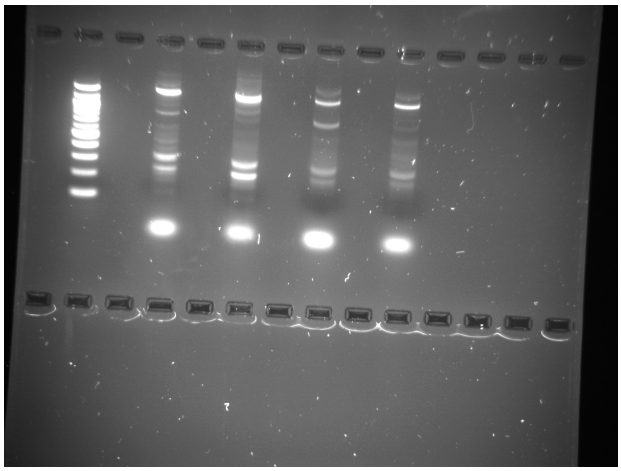

Annealing of forward anchor and reverse fluorescent anchor

Annealing of primers Protocol (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/etr-pMAZ6X2t-annealing-of-primers/edit)

2% Gel of cleaned G, D and to

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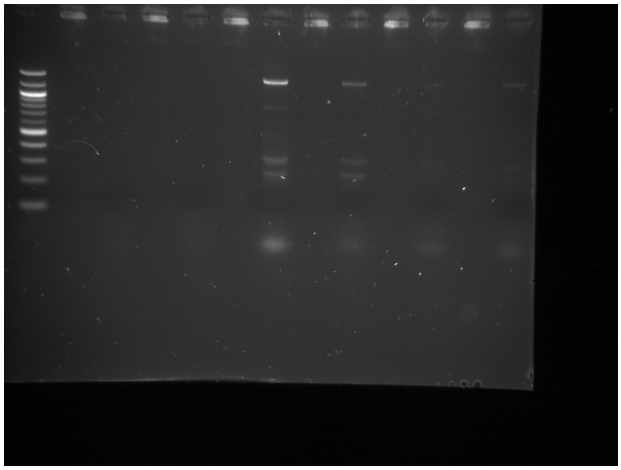

TUESDAY, 26/7

2% Gel of uncleaned, cleaned and digeste uncleaned and cleaned Good and Day

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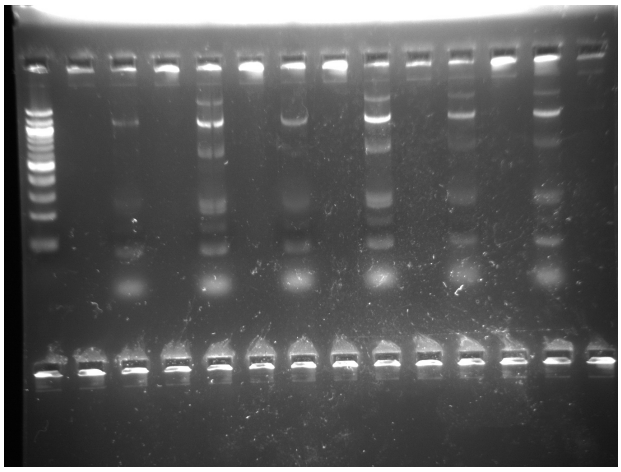

WEDNESDAY, 27/7

Miniprep of "There AB" and "Address00 BA"

Miniprep Protocol (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/etr-CfBQcrfj-miniprep/edit)

FRIDAY, 29/7

New binding assay with fluorescent anchor

Beads binding assay protocol (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/prt-1i8GoUud-bead-anchor-binding-efficiency-assay-1/edit)

Plate reader results:

Gain 1000:

Table6

	A	B	C	D	E	F	G
1		Old Protocol	20 µl beads	30 min incubation	25.5 µl DNA	2 wash	75 C incubation
2	Rep.1	2546	4081	1368	4108	3122	7001
3	Rep.2	2571	4083	1386	4130	3138	7059
4	Rep.3	2542	4054	1377	4109	3103	7007
5	Average	2553	4073	1377	4116	3121	7022

Gain 1500:

Table7

	A	B	C	D	E	F	G
1		Old Protocol	20 µl beads	30 min incubation	25.5 µl DNA	2 wash	75 C incubation
2	Rep.1	33021	52569	17906	53637	40464	91446
3	Rep.2	33005	52872	17909	53539	40725	91420
4	Rep.3	32945	52785	17825	53501	40844	91469
5	Average	32990	52742	17890	53559	40691	91445

Better results: elution buffer + 75 C Incubation, more DNA and less beads

MONDAY, 1/8

Resuspension of gBlocks (Dps)
-followed protocol on IDT specification sheetsì
DpsG made up to 50 µl (10 ng/ml)
DpsMP1 made up to 100 µl (10 ng/ml)

Digestion of Linearized Plasmid backbone psb1c
-found in distribution kit
-followed iGEM protocol
Enzyme mastermix:
- 5 µl CutSmart
- 0.5 µl EcoRI-HF
- 0.5 µl pstI-HF
- 18.5 µl H2O
= 25 µl for 5 reactions

Digestion of psb1c3
- 4 µl plasmid
- 4 µl MasterMix
made up to 20 µl with 1x buffer
1.2 µl buffer and 10.8 µl water
digested at 37 C for one hour, then left at room temperature overnight

Digestion of Dps
-2 µl EcoRI-HF
-2 µl PstI-HF
-20 µl buffer
-25 µl DpsG (0.25 ng) or DpsMPI (0.5 ng)
make up to 200 µl with water

Clean-up digest BabbleBrick plasmid
-used gel extraction kit to remove BabbleBricks and retain plasmid backbones
-step 9 -> 30 µl buffer EB
-put in freezer

TUESDAY, 2/8

Clean up of digested DpsG, DpsMPI and pSB1C3 using gel extraction kit

Insertion of Dps into pSB1C3

- 2 µl plasmid
- 2 µl T4 DNA ligase buffer
- 1 µl T4 DNA ligase
- 2 µl DpsG or DpsMPI
- 13 µl of H2O

left at room temerature

Transformation of plasmid into competent cells

Transformation (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/prt-VAC2Frf2-transformation-of-competent-cells-/edit)

PCR of "Good" and "Day" with new primers

PCR (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/prt-BaQRCshG-pcr/edit)

Assembly of 00AB and 00BA

1. Phosphorylation of anchor, words and terminator
   Phosphorylation (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/etr-DkqfiCsx-phosphoryation/edit)

2. Add 25 µl of anchor to 25 µl of AB word, then add 1 µl of igase and 20 µl of ligase buffer. Leave for 10 min at room temperature
3. Heat inactivation at 65 C for 10 mins
4. Add 20 µl of beads and wash them twice using 100 µl buffer
5. Add anchor plus word solution to the beads
6. Shake for 10 min at 900-1000 rpm at room temperature
7. Collect the supernatant
8. Repeat washing
9. Add 10 µl of next word, 20 µl of ligase buffer, 10 µl of wash buffer and 1 µl of ligase
10. Repeat steps 6, 7 and 8
11. Repeat step 9 for the terminator as well as 6, 7 and 8
12. Add 30 µl of Elution Buffer and melt at 75 C for 10 min

WEDNESDAY, 3/8

Annealing of primers

Annealing of primers (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/etr-pMAZ6X2t-annealing-of-primers/edit)

New assembly varying the number of words (6) and adding more word DNA (25 µl instead of 10 µl)

Gel of PCR from 2/08

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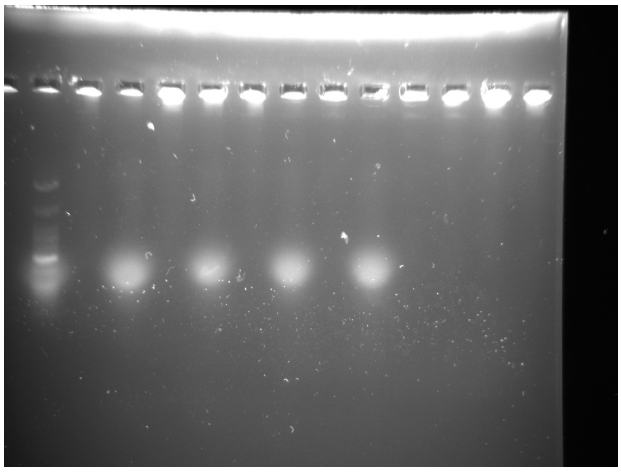

Beads binding assay using 25 µl of Fluorescent anchor, 25 µl of non-fluo anchor and 50 µl of Fluorescent anchor
Nanodrops result

Table8

	A	B
1	nAnchor 25 µl	68.4 ng/µl
2	FluoAchor 25 µl	39.5 ng/µl
3	FluoAnchor 50 µl	54.2 ng/µl

Transformation of BabbleBrick p-AB0 1 and 2, p-BA0 1 and 2

Resuspension of Biobricks:

- GFP generator
- recA (amp plate)
- chl cassette
- RBSI
- RBS2
- TERMI (to)
- CPFI (J32001)
- CPF2 (cons prom)

Resuspension of BioBricks (https://benchling.com/edinburgh_igem_team/f/f7oUXXpC-protocols/etr-3eeNjejh-resuspension-of-biobricks-igem-protocol/edit)

THURSDAY, 4/8

Assembly

3% agarose gel of Good and Day pcr

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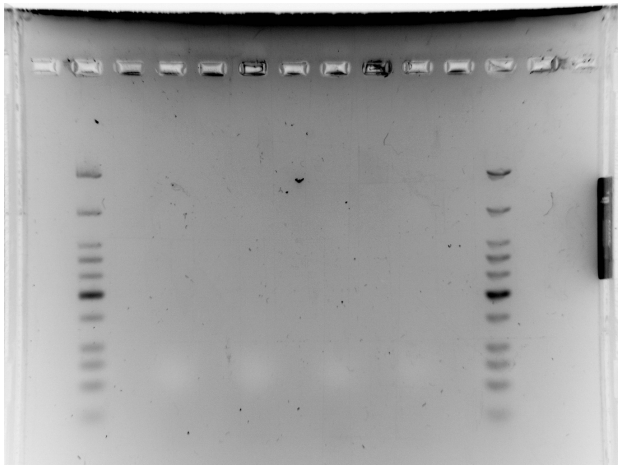

PCR of Good and Day with new primers

FRIDAY, 5/8

3% Gel for Good and Day and 0.8% gel for Chlo cassette

Annealing of anchor primers

Beads binding assay calibration curve

12 different anchor solutions were prepared, each having a final volume of 25 µl

Table9

	A	B	C	D
1	Tube	Final Concentration (µM)	Volume (µl)	Amount (nmol)
2	n-Anchor	0.1	25	0.0175
3	n-Anchor	1.05	25	0.02625
4	n-Anchor	1.4	25	0.035
5	n-Anchor	2.1	25	0.0525
6	n-Anchor	2.8	25	0.07
7	n-Anchor	3.5	25	0.0875
8	Fluo-Anch	0.7	25	0.0175
9	Fluo-Anch	1.05	25	0.02625
10	Fluo-Anch	1.4	25	0.035
11	Fluo-Anch	2.1	25	0.0525
12	Fluo-Anch	2.8	25	0.07
13	Fluo-anch	3.5	25	0.0875

MONDAY, 8/8

Transformation of word primers

Clen up of the PCR of the Chlo cassette

Inoculation

TUESDAY, 9/8

Assembly

Inoculation of Dps parts

Beads binding assay calibration curve

WEDNESDAY, 10/8

Annealing of primers

PCR of BabbleBricks AB 1.5 and BA 1.5 with new primers (the same primers for both libraries)

Assembly

Digestion of parts

Table10

	A	B	C
1	Digest Mix A (4x)	Digest Mix B (4x)	Digest Mix C (4x)
2	5 µl cutsmart (20)	5 µl cutsmart	5 µl cutsmart
3	0.5 µl EcoRI-HF (2)	0.5 µl XBaI	0.5 µl EcoRI-HF
4	0.5 µl SpeI (2)	0.5 µl PstI	0.5 µl PstI
5	19 µl dH2O (76)	19 µl dH2O	18.5 µl dH2O

Inoculate for 30 min (37 C) , heat kill for 20 min (80 C)

100 ng part, 4 µl Mix, 8 µl total reaction

Dephosphorylation of digested plasmid backbone
- put in 1 U (1 µl) of enzyme diluted in cutsmart buffer to the heat killed digestion
- fun product through gel extraction kit to clean up

Ligation
3 µl dephosphorylated, digested backbone (25 µl)
2 µl part A
2 µl part B
1 µl ligase buffer

0.5 µl ligase
dH2O to 10 µl

Transformation
the usual, except the incubation at 37 C went for an hourd and a half and we used 5 µl of plasmid

THURSDAY, 11/8

3% gel of Good and Day PCR

Gradient PCR

Preparation of 14% Polyacrylamide gels
final volume of 10 ml
3.34 ml dH2O
1 ml 10x TBE
1 ml 80% Glycerol
4.66 30% Acrylamide
6.4 ng APS
TEMED 25 µl

Inoculation:
-DpsG
-DpsMPI
-B0030
-GFP gen
-PrecA

Ligation:
- 1+2. DpsG + GFP
- 3+4. DpsMPI + GFP
- 5+6 Promoter+RBS

FRIDAY, 12/8

Miniprep of the Inoculation done on thursday

New assembly
Six assembly were done increasing the amount of word to 25 µl, decreasing the ligase buffer to 10 µl and the enzyme to 0.5 µl. The waiting time was also decreased to 5 min. The 3% gel showed two bands. They were cut out and gel extracted using the gel extraction kit. The samples were nanodrop.

Table11

	A	B	C	D
1	Unspecific band	Tubes 1 2 3	BabbleBlock Band	Tubes 1 2 3
2	Rep.1	6.2 5.4 6.1	Rep.1	7.1 6.1 7.8
3	Rep.2	7.4 9.4 7.0	Rep.2	5.9 6.6 7.9
4	Rep3	7.3 8.1 9.9	Rep.3	10.3 11.5 11.1
5	Mean	6.96 7.63 7.67	Mean	7.77 8.07 8.93

MONDAY, 15/8

PCR of pBA and pAB (1.5) at 52 C and 56 C

Digestion of PrecA , GFP and pSB1k3, ligation and transformation

Digestion of pSB1C3 (2), pSB1K3 and pSBIA3 (2)_(2)= 8 µl digested plasmid

Dilution of CIP phosphatase

Inoculation of Dps + GFP, and Promoter + RBS

Insertion of gel extracted BabbleBlock

Transformation of BabbleBlock

TUESDAY, 16/8

Inoculation of white colonies from the BabbleBlock transformation

New insertion of BabbleBlock with double DNA amount

Annealing of phosphorylated primer words (first sentence of Mary Queen's letter)

Gradient PCR for pBA1 (52 C to 67 C)

WEDNESDAY, 17/8

Insertion of BabbleBlock from the gel and transformation

Miniprep of the previous inoculation of BabbleBlock

Assembly of the first sentence of Mary's Queen letter

Annealing of primers

Miniprep of PrecA+GFP inoculation

Inoculation of J23100-B0030-Dps (x) constructs

Prepared mastermix for 2A assembly

Digested pSB1C3 for 2A assembly

SUNDAY, 21/8

Assembly (Final protocol)

Insertion and transformation of BabbleBlockk from gel

Inoculation and miniprep of BabbleBlock

MONDAY, 22/8

Gel of the assembly of the first sentence of the letter

TUESDAY, 23/8

PCR of AB and BA with new primers at 59 C

2, 4, 6, 8, and 10 words assembly

WEDNESDAY, 24/8

3% gel for the assemblies with different numbers of words

THURSDAY, 25/8

Annealing and Insertion of words and anchor primers

Assembly with 2, 4 and 10 words

FRIDAY, 26/8

Assembly with 2 and 4 words but with New Terminator

MONDAY, 29/8

Gel extraction of 2 and 4 words assembly bands

Miniprep of BabbleBlocks and preparation of 5 fmol/µl solutions

Insertion ligation of BabbleBlocks

THURSDAY, 1/9

PCR of "Good" using new final primers

Gel of PCR products

Transformation of competent cells.

Introduction

Best done with TOP10cells (Each aliquot contains 200ul).

Depending on the DNA you are transforming (i.e. a reaction or a plasmid) consider how much to use. Below is the protocol for transformation of plasmid.

The ratio of DNA to cells should not be over 10%, e.g. 100ul Cells, 10ul DNA.

Materials

- plasmid DNA
- competent TOP10 cells/competent DH5alpha cells
- SOC media/LB media
- correct antibiotic plate

Procedure

Using TOP 10 cells

1. Defrost cells on ice. Take 100ul of competent TOP10 cells
2. Add 1ul of plasmid DNA
3. Vortex and put on ice for 30min (turn on waterbath to 42*C and 37*C)
4. Heat shock at 42*C in a waterbath for 30secs
5. Return to ice for 30sec
6. Add 1ml of SOC media
7. Incubate at 37*C in waterbath for 1hr
8. Plate 100ul on to the correct antibiotic plate
9. Spin at 1000g the rest, remove most of media, reusupend in approx 100ul and plate onto another plate of the correct antibiotic
10. Put plates to 37*C overnight, or on the bench over the weekend.

Using DH5alpha cells

11. Defrost cells on ice. Take 200ul of competent DH5alpha cells
12. Add 2ul of plasmid DNA
13. Vortex and put on ice for 30min (turn on waterbath to 42*C and 37*C)

14. Heat shock at 42*C in a waterbath for upto 1min
15. Return to ice for 5min
16. Add 1ml of LB media
17. Incubate at 37*C in waterbath for 1.5-2hrs
18. Plate 50ul on to the correct antibiotic plate
19. Spin at 1000g the rest, remove most of media, reusupend in approx 100ul and plate onto another plate of the correct antibiotic
20. Put plates to 37*C overnight, or on the bench over the weekend.